The Role of *egl-38*/Pax in a *C. elegans* Hindgut Infection

Honors Research Thesis

Presented in partial fulfillment of the requirements for graduation with honors research distinction in Molecular Genetics in the undergraduate colleges of The Ohio State University

by

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Introduction

A cell's identity is determined by the set of protein and RNA molecules functioning in the cell. Differences between the sets of protein and RNA molecules present in two different cells can explain the differences between their shape, function, response to signals and even differences between their interactions with pathogens. All cells in a multicellular organism have the same DNA content, and so a specific cell must regulate which genes are expressed in order to take on the correct cell identity. Transcription factors, and the gene regulatory networks they form, are important in coordinating the regulation of gene expression and thus determining cell identity. This work focuses on Pax genes, which are a class of transcription factors important in animal development.

The defining characteristic of a Pax protein is the Paired box binding domain (Figure 1, PD). This domain consists of two helix turn helix subdomains which bind to specific DNA sequences and are connected by a flexible polypeptide linker (Xu, 1999). Some members of the Pax family also contain a third helix turn helix Homeobox DNA binding domain (Figure 1, HD). Another gene regulatory motif is the octapeptide repeat, which interacts with the Groucho family of co-repressors to repress target gene transcription (Chi, 2002). Furthermore, a predicted transcription activation domain (Figure 1, TD) at the C-terminal end of the polypeptide has been shown to function in transcriptional activation of target genes (Dorfler, 1996). The multiple DNA binding domains along with the presence of both transcriptional activation and repression domains illustrate that Pax genes have the potential to be more than just a simple on or off switch for a strictly defined group of target genes.

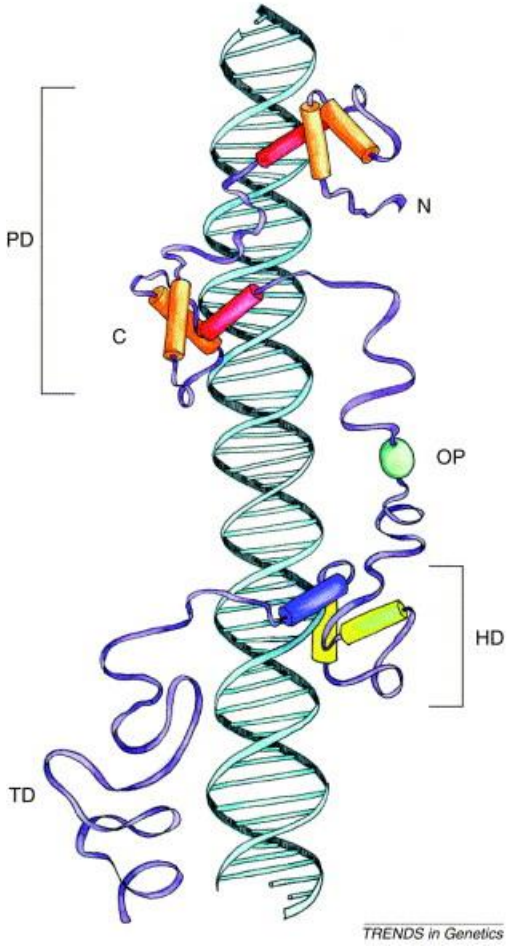

Figure 1.) Pax protein bound to its target DNA sequence. Abbreviations: PD – Paired domain; OP – Octapeptide repeat; HD – Homeodomain (not present in EGL-38); TD – Transcription activator domain. Chi, 2002

Considering the complexity of Pax proteins, it is not surprising that the sequence a specific Pax protein binds to is relatively degenerate. One explanation for this degeneracy is that a physical interaction between Pax and other cellular proteins can alter Pax-DNA sequence recognition. (Kondoh, 2010, Chi, 2002). As a result, the set of Pax target genes within an organism is dependent on the cellular context.

As a transcription factor, Pax's biological function is determined by the function of its target genes. *in vivo* ChIP experiments have identified that the largest categories of Pax target genes encode transcription factors and genes involved in signal transduction processes (Blake, 2008). This agrees with the well characterized role played by Pax genes in developmental

processes such as determining cell identity and coordinating organogenesis. The importance of Pax in these processes is best illustrated in Drosophila eye development. The Drosophila Pax homolog *eyeless* activates expression of a number of molecular cascades and eye specific proteins that are necessary for eye development. Furthermore, ectopic expression of *eyeless* in imaginal discs not associated with eye development will lead to the formation of an ectopic eye. The fact that Pax expression is sufficient for eye formation has led many people to refer to it is a master regulator of organogenesis and suggests that it is capable of regulating a large number of biological functions.

Pax homologs in *C. elegans* also play an important role in development. *egl-38* encodes an ortholog of the mammalian Pax2/5/8 gene family (Chamberlin, 1997). *egl-38* contains the paired box binding domain and octapeptide repeat motif, but does not contain the partial homeobox binding domain seen in the mammalian Pax2/5/8 gene class. Loss of function mutations of *egl-38* are lethal, but multiple non-null hypomorphic alleles are viable. Analysis of animals homozygous for these non-null hypomorphic alleles have demonstrated that *egl-38* is necessary for proper formation of the egg laying system and the hindgut (Chamberlin, 1997).

Figure. 2 A Diagram of the *C. elegans* hindgut.

The hindgut is made up of rectal epithelial cells that are arranged into three concentric rings. The rectum passes through these rings of cells and it functions as a pathway for the excretion of waste. The rectal epithelial cells are important in secreting substances that help

create and maintain the collagenous cuticle that forms the walls of the rectum (Altun, 2009). *egl-38* is expressed in the rectal epithelial cells K, K', F and U and *EGL-38* activity is necessary for these cells to take on the correct cell identity. These defects in rectal epithelial cell identity can be seen in animals homozygous for the non-null hypomorphic allele *egl-38(sy294)*, which show abnormal cell morphology and gene expression patterns (Chamberlin, 1997; Sewell 2003).

Animals homozygous for another hypomorphic allele, *egl-38(n578)*, show a resistance to infection from the bacterial pathogen *M. nematophilum* (Gravato-Nobre, 2008). *M. nematophilum* is a coryneform bacteria that colonizes the post-anal cuticle and the walls of the rectum as seen in figure 3 (Hodgkin, 2000). It is interesting that *M. nematophilum* colonizes the rectum of *C. elegans*, but not other openings to the environment such as the vulva (Gravato-Nobre, 2008). One can easily imagine a situation where the activity of the underlying rectal epithelial cells alters the molecular properties of the rectum in such a way that *M. nematophilum* is able to specifically recognize then attach to the rectum and proliferate.

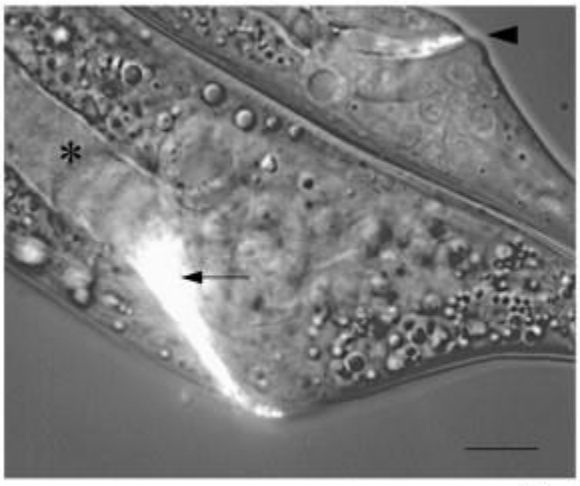

Figure 3.) *M. nematophilum* infection of *C. elegans* hindgut as visualized by SYTO 13 staining. Strong fluorescence can be seen in the lumen of the rectum (arrow). Hodgkin, 2000.

Infection by *M. nematophilum* is not lethal, but it does result in swelling of the tail region around the anus, which is termed deformed anal region or DAR (Hodgkin 2000). The Dar

response appears to result from intracellular swelling of rectal epithelial cells B, F, K and U (Gravato-Nobre, 2008). The swelling is mediated by a MAP kinase signal transduction cascade, although the cascade does not appear to be triggered by LET-60/Ras, which typically activates the cascade (Nicholas, 2004). The other major phenotype resulting from infection is delayed development. Animals grown on Plates containing a mixture of *M. nematophilum* and *E. coli* typically take 96 hours to reach adulthood, while animals grown on just the standard *E. coli* lawn only take 72 hours to reach adulthood (Gravato-Nobre, 2008). Another important indicator for *M. nematophilum* infection is the nucleic acid stain SYTO 13, which can be used to visualize the colonization of *M. nematophilum* in the hindgut (Hodgkin, 2000).

Table 1
***C. elegans* response to *M. nematophilum* infection**

Genotype	**SYTO 13**	**DAR**	**Rate of development**	Reference
Wild type	Present	Present	Delayed	Hodgkin, 2000; Gravato-Nobre 2008
egl-38 (n578)	Absent	Absent	Not Delayed	Gravato-Nobre, 2008; Helen Chamberlin unpublished data
bus-1 (e2678)	Absent	Absent	Not Delayed	Gravato-Nobre, 2008

M. nematophilum's ability to infect the hindgut of *C. elegans* is dependent on the genotype of *C .elegans*. Table 1 shows that animals with mutant alleles of *egl-38* and *bus-1* appear to be resistant to *M. nematophilum*. Both fail to show colonization through SYTO 13 staining and both lack the DAR and delayed development seen in infected animals (Gravato-Nobre, 2008). *bus-1* is a predicted integral membrane O-acyltransferase that is expressed in the rectal epithelial cells K, K', F and U. Because animals homozygous for the null *bus-1(e2678)* allele show no other obvious phenotypes besides resistance to *M. nematophilum*, it is difficult to

pinpoint the biological function BUS-1 is performing in the animal. But because the infection appears to be localized to the cuticle lining of the hindgut and because the rectal epithelial cells modify this cuticle, one likely function of BUS-1 is the modification of this cuticle. *M. nematophilum* could then recognize this modification, which would explain the infection's dependence on *bus-1*.

A previous mRNA microarray result showed that levels of *bus-1* mRNA are reduced in *egl-38(n578)* mutants. This result shows that *bus-1*expression is dependent on *egl-38*, but what has not been established is whether EGL-38 is directly regulating *bus-1*expression by binding to a *bus-1* enhancer or regulating *bus-1* through an indirect mechanism such as EGL-38 enhancing expression of a transcription factor that then activates *bus-1* expression. Either way, what is interesting about this relationship is that *bus-1* appears to be a physiological output of EGL-38 activity that is specific to rectal epithelial cells. Identifying the functional outputs of Pax genes and how these outputs are regulated is an important first step in understanding how Pax genes control larger biological functions like determining cell identity.

Results and Discussion

egl-38 dependence of *bus-1* expression

In order to confirm the expression pattern of *bus-1*, a *bus-1* reporter construct was made by cloning the 1500 base pair fragment of DNA located 5' to the start of the *bus-1* gene into a plasmid containing a GFP coding sequence. When this construct is placed *in vivo* as an extrachromosomal transgene, the 1500 base pair fragment will drive expression of the GFP protein, which can then be visualized. Figure 5 shows a typical animal containing this transgene. GFP fluorescence can be seen in the F, K, K' and U cells in most animals and some animals show fluorescence in P12.Pa and B.

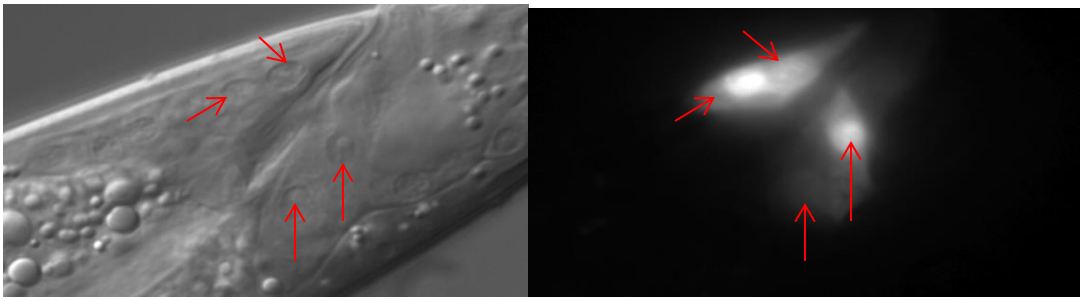

Figure 4.) A typical animal with the *C. elegans bus-1*::*GFP* reporter construct. Left: DIC image of the animal. Right: Image of GFP fluorescence in the same animal. From left to right, arrows indicate the rectal epithelial cells K, U, P12.pa, and F.

These reporters were then crossed into animals homozygous for *egl-38(n578)* or *egl-38(sy294)*. Figure 6 shows that both *egl-38(n578)* and *egl-38(sy294)* genetic backgrounds show a lower proportion of animals with *bus-1::gfp* expression. This confirms that *bus-1* expression is dependent on *egl-38*. It also confirms that *egl-38* is either directly or indirectly enhancing expression of *bus-1*.

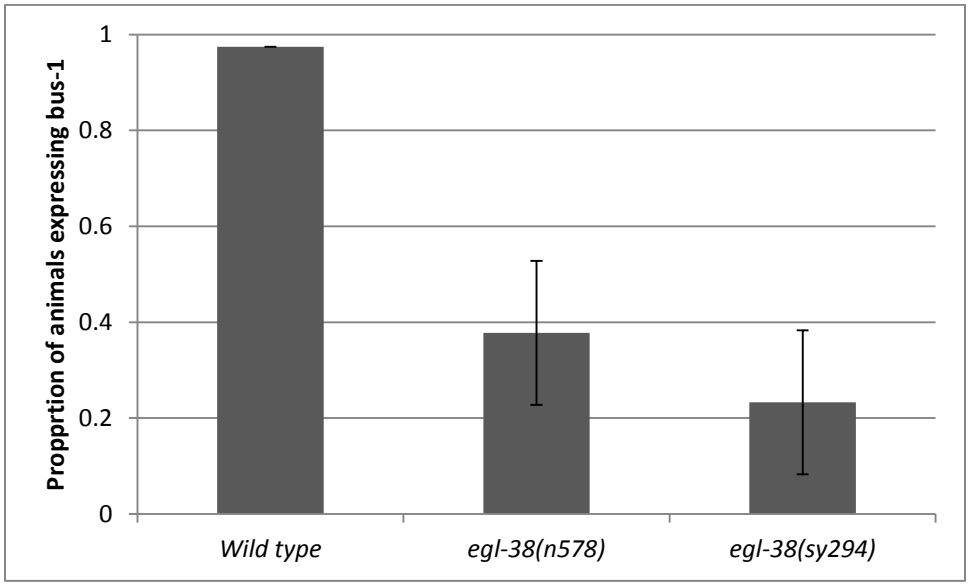

Figure 5.) Proportion of animals containing the *C. elegans bus-1::gfp* reporter transgene A in various genetic backgrounds. An animal with GFP in either K, K', F or U was scored as positive. Error bars represent 95% confidence intervals as determined by a normal approximation interval for proportions. N>30 for all genotypes.

BUS-1 rescue in *egl-38(n578)*

Due to the complexity of host- pathogen interactions, there is likely more than one molecular feature unique to the hindgut that *M. nematophilum* recognizes. Furthermore, bus-1 expression is not completely lost in *egl-38(n578)* animals. Therefore it is unlikely that the reduced bus-1 expression in *egl-38(n578)* animals is the sole reason for the resistance to infection seen in *egl-38(n578)* animals. We hypothesize that there are a set of egl-38 dependent genes expressed in the rectal epithelium that are important for infection by M. nematophilum.

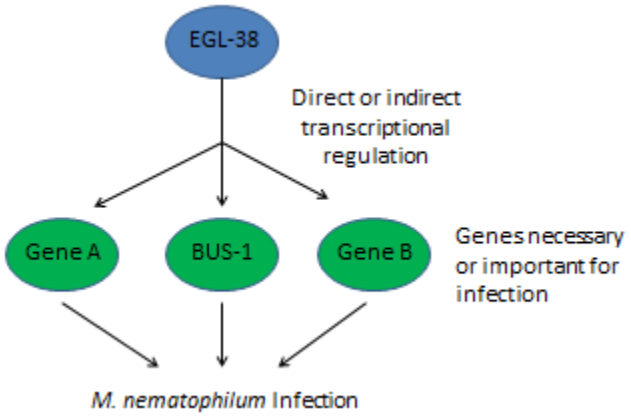

Figure 6.) Diagram of the hypothesized role of *egl-38* in infection. Gene A and Gene B represent hypothetical *egl-38* dependent genes that are necessary or important for M. nematophilum infection. The altered expression of these hypothetical genes and *bus-1* in *egl-38(n578)* mutants could fully explain the *egl-38(n578)* resistance to infection phenotype.

To test this, a *bus-1* rescue construct was made. This rescue construct was created by cloning a fragment of the *egl-5* promoter upstream of the *bus-1* coding sequence. The stop codon of the *bus-1* coding sequence was not included in the construct and instead an eighteen base pair linker was used to connect the *bus-1* coding sequence with a sequence coding for GFP. This resulted in the construct shown in figure 8 that has the *egl-5* promoter driving expression of a BUS-1 protein tagged with GFP.

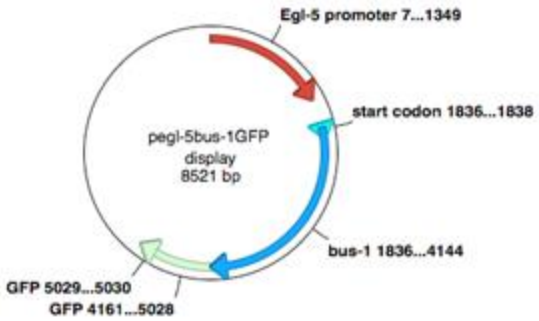

Figure 7.) Diagram of the *egl-5::bus-1* rescue construct.

pLG7, the *egl-5* promoter fragment, drives expression in K, F, U, P12.pa, B and the posterior body wall muscle (Teng, 2004). *egl-5* encodes a Hox gene. The expression patterns of Hox genes are set up early in development to define positioning along the anterior/posterior axis of the animal and are unlikely to be affected by *egl-38*. Thus, the *egl-5* promoter allows the construct to express *bus-1* even in an *egl-38(n578)* genetic background. Unfortunately, the fluorescence signal from the *bus-1* tagged GFP is very weak, which makes ensuring that there is no change in expression between wild type and *egl-38(n578)* animals difficult to confirm. A weak signal from the rescue construct was seen localizing to cells of the rectal epithelium in a few animals of both wild type and *egl-38(n578)* genetic background.

Figure 8 shows the response of animals with various genotypes after they were allowed to develop on lawns of M. nematophilum. The response to infection was measured by the proportion of animals displaying a DAR phenotype.

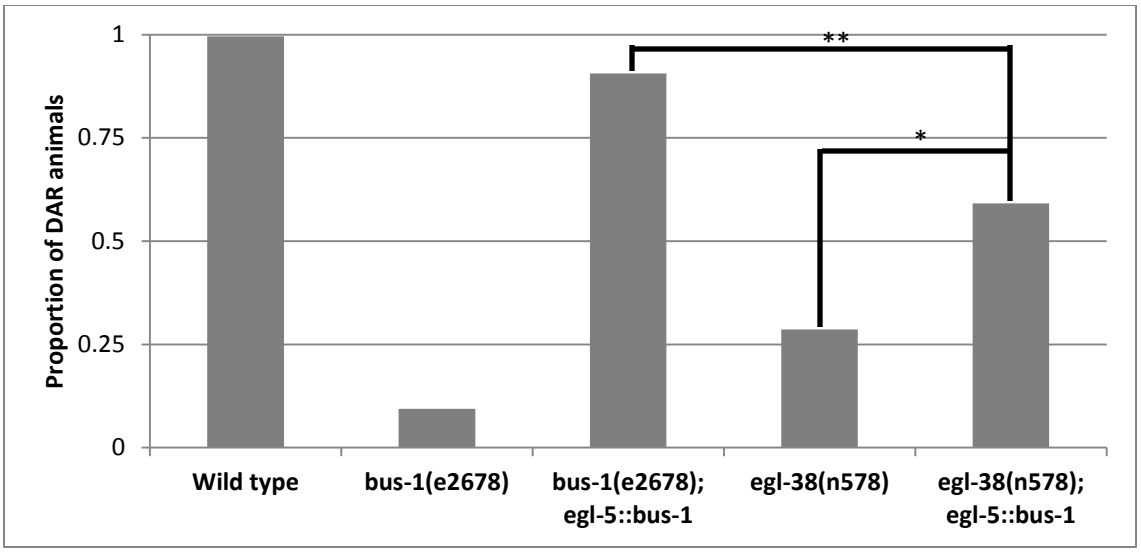

Figure 8.) The proportion of animals displaying a DAR phenotype after developing on mixed lawns of OP50 and *M. nematophilum*. * = one tailed two proportion Z test showed the proportion of *egl-5::bus-1;egl-38(n578)* animals with DAR is significantly greater than *egl-38(n578).* ** = Two tail two proportion Z test showed a significant difference between the proportion of *egl-5::bus-1;egl-38(n578)* animals with DAR and *egl-5::bus-1;bus-1(e2678).* P<.01

241/242 wild type animals had a DAR phenotype while only 43/459 animals homozygous for the null *bus-1(e2678)* had the phenotype. This is consistent with similar experiments performed by Gravato-Nobre. 586/647 animals with the *bus-1(e2678)* genetic background containing the transgenic *egl-5*::*bus-1* rescue construct displayed a DAR phenotype. This result demonstrates that the *bus-1* rescue construct is able to nearly restore the animal to a wild type infection phenotype. 170/595 *egl-38(n578)* animals showed a DAR phenotype, which is a greater proportion than *bus-1(e2678).* One reason for this difference may be due to the fact that a low proportion of *egl-38(n578)* animals are DAR even when they are grown on the typical E. coli lawns. A DAR phenotype was displayed by 318/538 *egl-38(n578)* animals containing the transgenic rescue construct. There is a statistically significant larger proportion of *bus-1* rescued *egl-38(n578)* animals with DAR than non-rescued *egl-38(n578).* But there is also a statistically significant difference between the proportion of *bus-1* rescued *egl-38(n578)* animals with DAR and *bus-1* rescued *bus-1(e2678)* animals with DAR.

The nearly wild type proportion of *bus-1* rescued *bus-1*(e2678) animals suggests that the *egl-5*::*bus-1* rescue construct is able to produce enough functional BUS-1 protein to compensate for the absence of functional BUS-1 protein found in animals with the null *bus-1(e2678)* allele. If reduced *bus-1* expression in *egl-38(n578)* animals is the sole reason for the resistance to infection seen in these animals then one would expect the *bus-1* rescued *egl-38(n578)* animals to have the nearly wild type proportion of DAR animals seen in the *bus-1* rescued *bus-1(e2678)* null animals. The experimental data show that returning *bus-1* expression in *egl-38(n578)* animals is unable to fully restore the proportion of DAR animals predicted by the *egl-5*::*bus-1*;*bus-1*(e2678) control. This suggests that reduced *bus-1* expression in *egl-38(n578)* animals is not the sole reason for the resistance to infection phenotype seen in *egl-38(n578)* animals. But restored *bus-1* expression in *egl-38(n578)* did make the *egl-38(n578)* genetic background less resistant to infection. This suggests that reduced *bus-1* expression in *egl-38(n578)* animals is partially responsible for the *egl-38(n578)* resistance to M. nematophilum phenotype.

The experimental data only suggest that reduced *bus-1* expression in *egl-38(n578)* is not the sole reason for *egl-38(n578)* resistance. To rigorously prove this hypothesis, an experiment must confirm that the *egl-38(n578)* genetic background does not alter the expression of *bus-1* in the *egl-5*::*bus-1* rescue. One such experiment would be a quantification of *bus-1* mRNA produced by the transgene in *egl-5*::*bus-1*;*bus-1*(e2678) and *egl-5*::*bus-1*;*egl-38(n578)* animals.

Nevertheless, the rescue results still suggest that lowered *bus-1* expression cannot fully explain the *egl-38(n578)* resistance to M. nematophilum phenotype. If this is the case, then other *egl-38* dependent genes must be necessary or important to create an environment in the *C. elegans* hindgut that M. nematophilum is able to recognize and is suitable for proliferation of the bacteria. This allows for the interesting possibility that *egl-38* controls a battery of genes in

rectal epithelial that help to create an environment that M. nematophilum can specifically recognize. This battery of genes could be a functional output of EGL-38 activity; an output that determines cell identity.

Deletion analysis of the *C. elegans bus-1* regulatory region

Understanding what cis-regulatory sequences control the expression of *bus-1* can give clues as to what trans-acting factors regulate *bus-1* expression. Identification of these trans-acting factors would lead to an understanding of how EGL-38 regulates the *bus-1* and potentially other *egl-38* dependent genes.

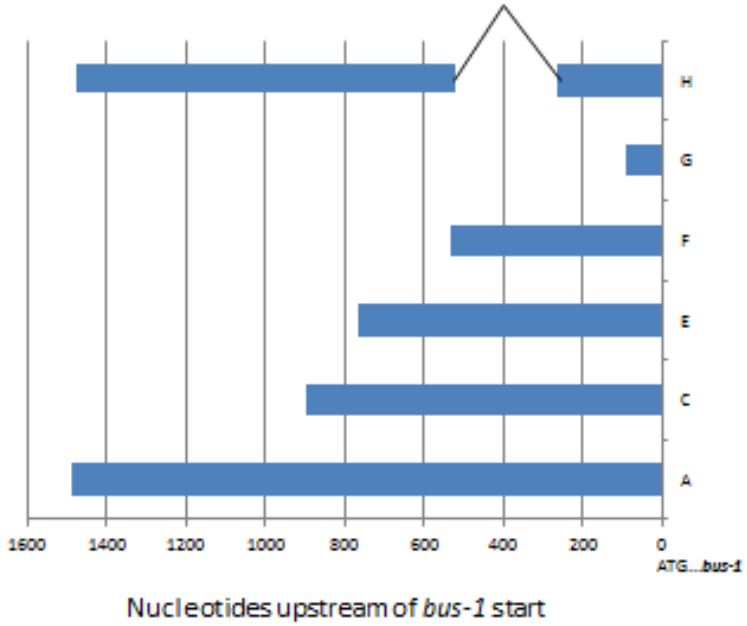

Figure 9.) The various DNA fragments upstream of the *bus-1* gene that were used to drive expression of GFP in the deletion analysis *bus-1* reporter constructs are represented by the blue bars. The X-axis represents the location X number of nucleotides upstream of the start codon of the *bus-1* gene. The coding sequence of bus-1 is to the right of 0. Reporter construct H fuses together the two fragments connected by the line.

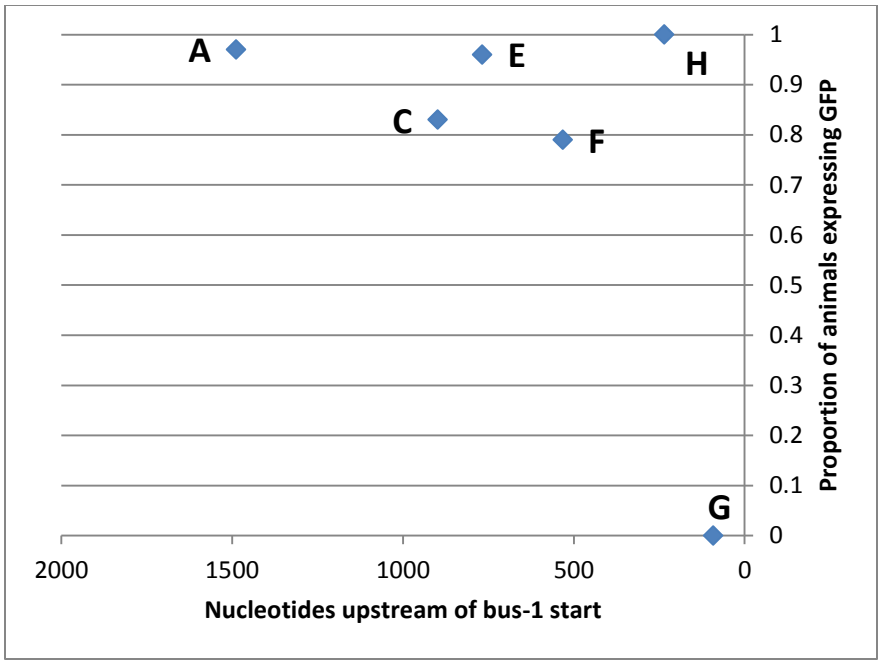

Figure 10.) Results of deletion analysis. The Y-axis represents the proportion of animals showing expression in either K, K', U or F. The X-axis represents the number of nucleotides upstream of the start codon of the bus-1 gene. Each point represents the proportion of animals containing the deletion reporter construct referenced in figure 9. The X value represents the location X nucleotides upstream of the start codon that drives expression of the GFP reporter.

A series of *bus-1* reporter transgenes were created to identify what sequence upstream of the *bus-1* gene was necessary for expression of *bus-1* in the rectal epithelial cells. Figure 9 shows a diagram of the DNA fragments that were used to drive GFP expression in the *bus-1* reporter transgenes. If a necessary cis-regulatory sequence is not present in one of the reporter transgenes then that transgene will not express GFP in the rectal epithelial cells. Figure 10 illustrates that expression is present in the F and H constructs, which does not contain sequences upstream of the first 235 nucleotides. But expression is lost in the reporter transgene G, which contains only the first 92 nucleotides. It can be concluded that the *C. elegans* cis-regulatory regions necessary for expression of *bus-1* are between 92-235 nucleotides upstream from the start codon of the *bus-1* gene.

EGL-38 is one potential trans-acting factor that may regulate *bus-1*. Another member of the lab used an electrophoretic mobility shift assay to test whether EGL-38 could directly bind to

oligonucleotides from the region 92-235 nucleotides upstream of *bus-1*. The results showed that EGL-38 does not bind to that region *in vitro* no binding, although this does not eliminate the possibility that EGL-38 is directly regulating *bus-1 in vivo*.

Regulation of *bus-1* in *C. briggsae*

C. briggsae and *C. elegans* are closely related nematode species. Important *bus-1* cis-acting regulatory sequences should be conserved between *C. elegans* and *C. briggsae*. Gravato-Nobre identified a *C. briggsae* gene with significant sequence similarity to *C. elegans bus-1* in both coding and upstream regions. They confirmed that this *C. briggsae* gene is the *C. elegans* homolog by rescuing the *bus-1(e2678)* mutant phenotype with this *C. briggsae* gene. This gene will be referred to as *C. briggsae bus-1*.

To confirm that *C. briggsae bus-1* exhibits the same expression pattern as *C. elegans bus-1*, a reporter construct was created that used a *C. briggsae* upstream region, which corresponds to the 235 nucleotide upstream region in *C. elegans bus-1*, to drive expression of GFP. Expression was seen in the rectal epithelial cells K, K', U and F along with B and P12.pa., when this *C. briggsae bus-1* reporter construct was placed in *C. elegans*. Unlike the *C. elegans bus-1* reporter, the *C. briggsae bus-1* reporter also fluoresced in cells of the vulva.

Next, the *egl-38* dependence of the *C. briggsae bus-1* reporter was tested by crossing the transgene into an *egl-38(n578)* and *egl-38(sy294)* genetic background. Figure 11 shows that the *C. briggsae bus-1* shows reduced expression in the *egl-38(sy294)* genetic background. The reduction in expression is smaller than in figure 6, which uses a *C. elegans bus-1* reporter. Nevertheless, both *C. elegans* and *C. briggsae bus-1* reporters show the same pattern of expression in rectal epithelial cells and this expression is at least partially dependent on *egl-38*.

Therefore it is reasonable to search for conservation in the alignment of the two regulatory regions in order to find potentially important cis-regulatory sequences.

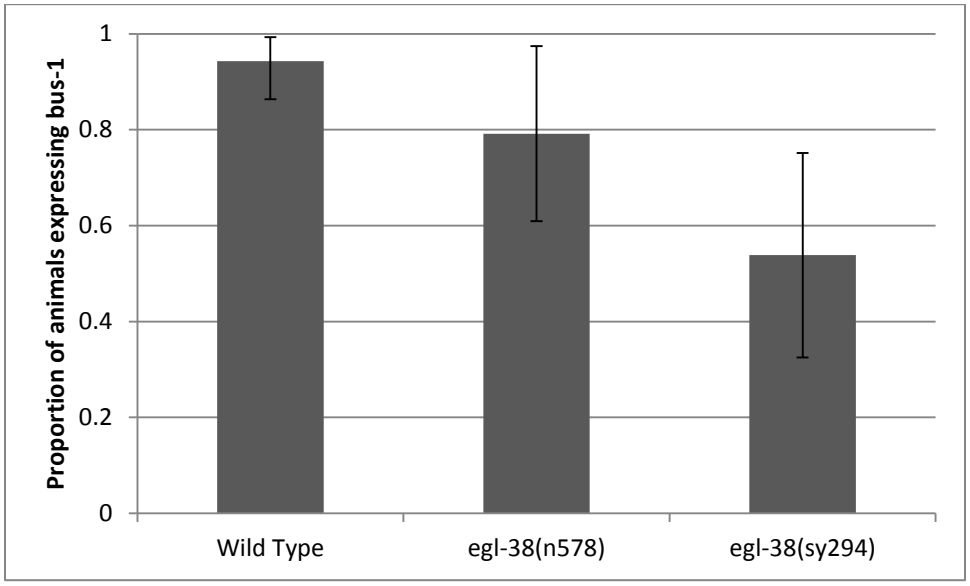

Figure 11.) Proportion of *C. elegans* animals containing a *C. briggsae bus-1::gfp* reporter that have GFP expression in either K, K' U or F. 95% confidence intervals calculated using the normal approximation interval for proportions.

Conservation between *C elegans* and *C. briggsae bus-1* regulatory regions

The alignment performed by the UCSC genome browser was used to identify areas of conservation between the upstream regions of *C. briggsae* and *C. elegans bus-1*. The search for conservation was restricted to the region 92-235 nucleotides upstream of the *bus-1* start, because it is the region necessary for rectal epithelial expression in *C. elegans*. Figure 12 shows this necessary region and blocks of conserved sequences are highlighted.

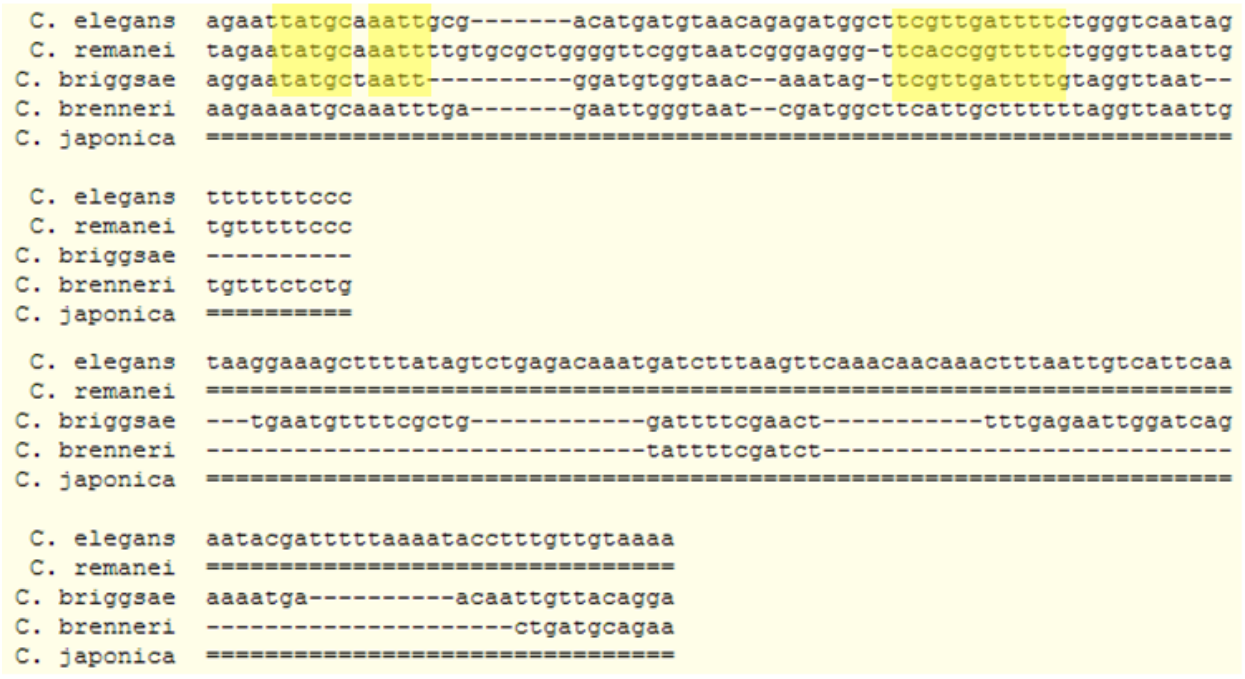

Figure 12.) UCSC genome browser alignment of the upstream sequences necessary for *bus-1* expression from multiple nematode species. From left to right the sequence is the 92 nucleotide upstream of *C. elegans bus-1* to the 235 nucleotide upstream of *C. elegans bus-1*. Highlighted blocks of sequence are conserved between *C. elegans* and *C. briggsae* (UCSC genome browser, *C. elegans* ChrV:9024853-9024767).

These conserved blocks could be important cis-regulatory sequences. In the future, the observation of a loss of hindgut expression following the site directed mutagenesis of one of these sites would experimentally show that it is a cis-regulatory sequence. But until that experiment is performed it is hard to predict which sites, if any, are cis-regulatory sequences. Furthermore, it is possible that sequences in the necessary region of *C. elegans* and *C. briggsae* that do not appear to be conserved may play similar cis-acting regulatory roles. As a result, future work should not focus exclusively on the conserved colored sequences.

Another approach for identifying the cis-acting regulatory sequences and the trans-acting factors important for *bus-1* regulation would be to use an algorithm that searches for consensus transcription factor binding sites. RNAi or mutant alleles could then be used to test whether the candidate trans-acting factors can alter expression of *bus-1*.

Future Directions

To better understand the functional outputs of EGL-38 activity, more hindgut specific *egl-38* dependent genes need to be identified. A good starting point would be to identify *egl-38* dependent genes that are important for M. nematophilum infection. Then, one could identify common trans-acting factors important for regulation of these genes and characterize how these trans-acting factors relate to *egl-38*. Knowledge of this relationship between egl-38, the trans-acting factors it regulates and functional outputs of EGL-38 activity will allow us to understand the mechanism of how *egl-38*'s regulation of specific target genes translates into the biological function performed by *egl-38* and other related Pax proteins

Materials and Methods

Construction of *C. elegans bus-1* reporter constructs A, C, E, F and G

A PCR reaction using purified *C. elegans* N2 genomic DNA as a template was run to amplify the appropriate upstream regulatory region used to drive GFP expression. Unique forward primers were used to amplify the appropriate fragment. Gel electrophoresis was used to confirm each product was of the appropriate size. XbaI and BamHI restriction sites on the end of the primers were digested and then the product was gel purified. The pPD95.69, which contains the GFP coding sequence and a CARB resistance gene, was digested using XbaI and BamHI then gel purified. The digested PCR product and pPD95.69 were ligated and transformed into competent DH5α bacteria. The bacteria were plated onto CARB plates and clones were cultured in LB + CARB overnight. Plasmids were extracted from the culture and diagnostic restriction digests were used to ensure the correct plasmid was isolated. These *C. elegans* bus-1 reporter construct plasmids were then column purified.

Construction of *C. elegans bus-1* reporter construct H

The *C. elegans bus-1* reporter construct A was used as a PCR template. Primers were designed to amplify the whole construct except for the "deleted" 265 base region indicated in figure 10. The NheI restriction sites at the end of the primers were cut and the digested PCR product was gel purified. The digested product was then ligated and from this point the procedure was identical to the one above.

Construction of *C. briggsae bus-1* reporter construct H

The exact same procedure used to create the *C. elegans bus-1* reporter construct was performed, except *C. briggsae* AF 16 genomic DNA was used as a template and the unique *C. briggsae* primers contained XbaI and SphI restriction sites.

Construction of the *egl-5::bus-1* rescue construct

A pLG7 plasmid template was used to amplify an 1823 base pair PCR product containing the egl-5 regulatory fragment and a *pes-10* basal promoter fragment, which drives expression in the rectal epithelial cells. HindIII and XbaI restriction sites, on the forward and reverse primer respectively, were digested and the product was gel purified. A pPD95.77 plasmid, which contains a sequence coding for GFP and CARB resistance, was also digested using XbaI and HindIII then gel purified. The digested PCR product and pPD95.77 plasmid were ligated and transformed into DH5α bacteria. The bacteria were plated onto CARB plates. Clonal colonies were picked and cultured in LB + CARB overnight. Plasmids were extracted and diagnostic digests were run to ensure the correct plasmid was isolated. These plasmids were then column purified.

The sequence containing the *bus-1* gene was amplified using PCR from *C. elegans* N2 genomic DNA. XbaI and KpnI restriction sites, on the forward and reverse primer respectively, were digested and gel purified. The pPD95.77 plasmid containing the egl-5 promoter, which is

described above, was digested using XbaI and KpnI then gel purified. These were ligated and transformed into DH5α bacteria. The bacteria were plated onto CARB plates. Clonal colonies were picked and cultured in LB + CARB overnight. Plasmids were extracted from the cultured DH5α and diagnostic digests were run to ensure the correct plasmid was isolated. These egl-5::bus1 rescue constructs were then column purified.

Creation of *transgenic* lines containing the *C.elegans* and *C. briggsae bus-1* reporter constructs

Injection mixtures containing 75 ng/µL of a *bus-1::gfp* reporter construct plasmid and 15 ng/µL of *unc-119(+)* DNA were created. These were microinjected into the mitotic gonads of a RH 10 *C. elegans* strain. RH10 animals are homozygous for *unc-119(e2498)* and display an uncoordinated phenotype. F1 progeny of the injected animals were screened for wild type movement. Lines of animals with stable transmission of the extrachromosomal transgenes, as determined by wild type movement, were established. *Transgenic* males were crossed with *egl-38(sy294* or *n578)/nT1; unc-119(e2498)/unc-119(e2498)*. Wild type moving hermaphrodites were selected and allowed to self-fertilize to create a homozygous strain.

Creation of *transgenic* lines containing the *egl-5::bus-1* rescue construct

Injection mixtures containing; 30 ng/µL of the *egl-5::bus-1* rescue plasmid, 15 ng/µL of the *unc-119(+)* DNA, and 50 ng/µL of PBII SK (genomic yeast DNA) were created. Microinjections and subsequent steps are the same as above.

Analysis of GFP reporter transgenes

Transgenic lines were grown on a standard lawn of OP50 bacteria at 20^{o}C. L4 animals were placed on slides containing M9 buffer and a small amount of Sodium Azide to anesthetize the animals. Animals on the slide were observed under high magnification Normarski microscopy. A cell with GFP fluorescence above a background level was counted as GFP

positive. Animals were scored positive for hindgut expression if either F, K K' or U were GFP positive.

M. nematophilum assay for DAR

10 L4 animals were placed on plates with a 9:1 ratio of OP50 to M. nematophilum CBX 102. The animals were allowed to propagate for five days. Animals ranging from L2 to adults were scored for the presence of DAR using a low magnification light microscope with 40 times magnification. Animals with any observable post-anal bump were counted as positive for the DAR phenotype.

At least three trials were performed for each genotype and no significant variation between trials was observed. The plates were coded to ensure that the individual scoring did not know the genotype of the worms. The data from all the trials of a genotype were combined to form the proportion of animals with DAR seen in figure 9.

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